Discrete and Hybrid Modelling of Gene Regulatory Networks

How to handle time in formal models ?

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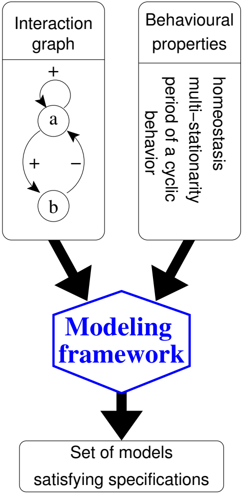
Outline

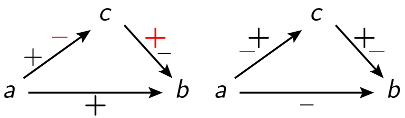
Introduction

- Modelling of genetic regulatory network
  - ⇒ deep understanding of how the components interact
  - ⇒ non obvious predictions on possible behaviours
- information about interactions increases
  ≠ kinetic data not available
- Parameter identification problem is crucial
- Qualitative models : the problem is easier
  ⇒ good compromise
- Importance of time in the dynamics of a system
- Qualitative models with time : Hybrid models

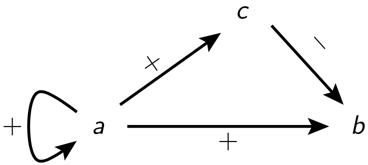


A feedforward loop controled by a positive auto-regulation

- Feedforward loop : one of the most common interaction patterns
  - Incoherent : the signs of both paths are different
  - 4 different patterns.



- We consider that the action of *a* does not change with time
- the simplest way : a functional positive loop on *a*
- Incoherent type 1 feedforward loop combined with a positive auto-regulation of *a*



Differential Framework

- with each variable v is associated a value $x_v \in \mathbb{R}$ (concentration)
- State a the system : $(x_v)_{v \in V}$
- Ordinary Differential Equation System :

$$\frac{dx_v}{dt} = F_v(x) - \lambda_v x_v \qquad \forall v \in \{1, 2, \ldots n\}$$

with

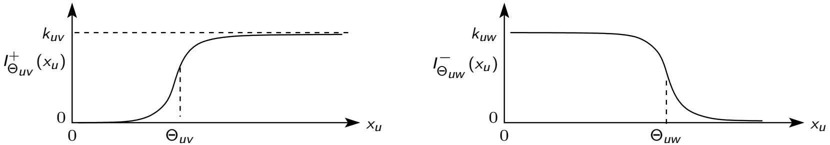
$$\begin{cases} \lambda_v \geq 0 & : \text{degradation coefficient} \\ F_v(x) & : \text{synthesis rate of variable } v \end{cases}$$

Often, synthesis rate is additive :

$$F_v(x) = \sum_{u \in G^-(v)} I(u, v) \quad \text{contribution of } u \text{ to the synthesis rate of } v$$

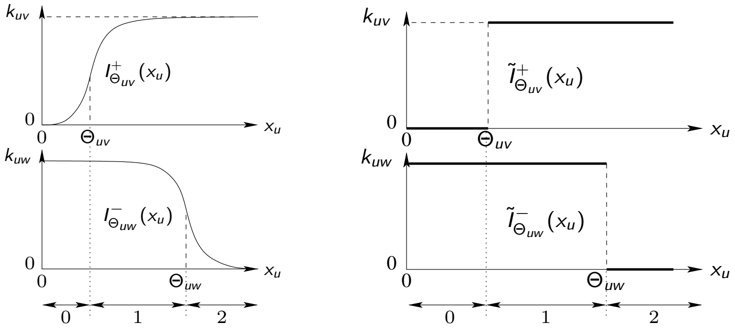
Synthesis rate

- Often, u has a quasi-null effect when below threshold θ_{uv} and a saturated effect when above $\Longrightarrow$ Sigmoïdal function



  - $x_u < \Theta_{uv}$: u is in too small quantity to regulate v
  - $x_u > \Theta_{uv}$, u does regulate v
- u helps v
  - if u is an activator of v and if $x_u > \Theta_{uv}$
  - if u is an inhibitor of v and if $x_u < \Theta_{uv}$
- **the absence of an inhibitor = the presence of an activator**
- Resources$(v) = \{$regulators of v contributing to the synthesis rate of $v\}$

Discretisation of phase space



$\tilde{I}^{+}_{\Theta_{uv}}(.)$ is not defined at Θ_{uv}

$\tilde{I}^{-}_{\Theta_{uw}}(.)$ is not defined at Θ_{uw}

synthesis rate is then : $$f_v(x) = k_v + \sum_{u \in \mathrm{resources}(v)} k_{uv}$$

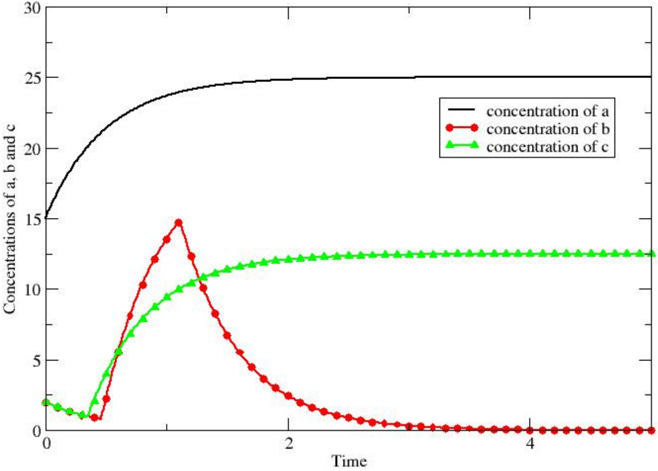
Regular domains (for all variable $u, x_u \neq \theta$)

- Equations are independant – for variable x_v : $\boldsymbol{x'_v = \mu - \lambda_v x_v}$
- Solution : $x_v(t) = \frac{\mu_v}{\lambda} - (\frac{\mu_v}{\lambda} - x_0^v).e^{-\lambda t}$
- The vector $(\frac{\mu_v}{\lambda_v})_v$ is the **focal point** of the domain
- Derivative : $x'_v(t) = (\frac{\mu_v}{\lambda} - x_0^v).e^{-\lambda t}$
  The sign of derivatives does not change
  $\Longrightarrow$ trajectories are **monotonous** on each axis.
- A particular case : $\lambda_v = \lambda, \forall v \in V$

$$\overrightarrow{v(0)} = ((\tfrac{\mu_1}{\lambda} - x_0^1), (\tfrac{\mu_2}{\lambda} - x_0^2), \ldots, (\tfrac{\mu_n}{\lambda} - x_0^n))^t$$
$$\overrightarrow{v(t_1)} = ((\tfrac{\mu_1}{\lambda} - x_0^1), (\tfrac{\mu_2}{\lambda} - x_0^2), \ldots, (\tfrac{\mu_n}{\lambda} - x_0^n))^t \times e^{-\lambda t_1} = \overrightarrow{v(0)}.e^{-\lambda(t_1)}$$

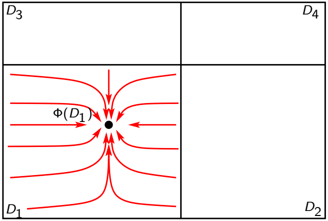
$\Longrightarrow$ trajectories are **rectilinear**

FFL controled by a positive auto-regulation



Discrete Modelling (R. Thomas & E.H. Snoussi)

1. *Taking into account only regular domains*
   - a domain corresponds to a *qualitative* state
   - frontiers are abstracted
     - if trajectories do not stay in such frontier : OK
     - if not : characteristic states or qualitative reasonning on differential inclusion ($\frac{dx_i}{dt} \in H(x)$)
2. *Taking into account only qualitative behaviors*



The focal point is in the current domain
Trajectories do not go out of the domain.
$\Rightarrow$ no exit

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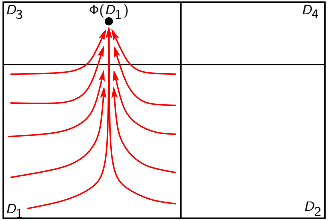

The focal point is in the domain D_3
All trajectories go out of the domain
$\Rightarrow$ in the north direction

Discrete Modelling (R. Thomas & E.H. Snoussi)

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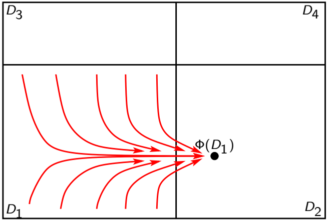
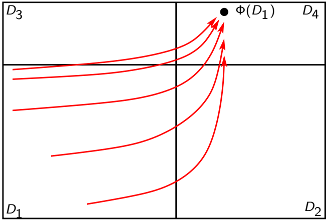


The focal point is in the domain D_2
All trajectories go out of the domain
$\Rightarrow$ in the east direction

Discrete Modelling (R. Thomas & E.H. Snoussi)

1. *Taking into account only regular domains*
   - a domain corresponds to a *qualitative* state
   - frontiers are abstracted
     - if trajectories do not stay in such frontier : OK
     - if not : characteristic states or qualitative reasonning on differential inclusion ($\frac{dx_i}{dt} \in H(x)$)
2. *Taking into account only qualitative behaviors*



The focal point is in the domain D_4
All trajectories go out of the domain
⇒ in the east direction
⇒ in the north direction

Discrete Modelling (R. Thomas & E.H. Snoussi)

- Parameters :
  - in the continuous framemork : degradation rates, synthesis rates
  - in the discrete framework : positions of the focal points

$$K_{v,\omega} = \text{coordinate } v \text{ of the focal point when } resources(v) = \omega$$

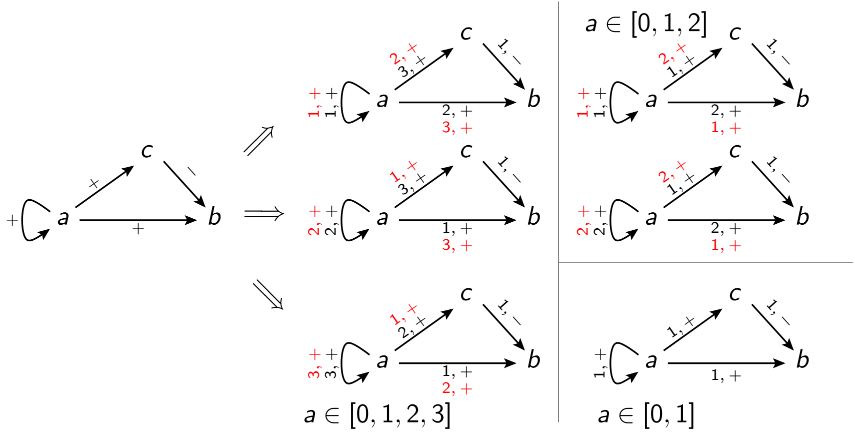
$\Rightarrow$ Finite (but often enormous) number of parameterizations to consider

Schema of modelling process

1. Construct the graph of schematic interactions
2. Associate with each "gene" a variable
3. Determine the number of levels of each variable
   (generally, the number of interactions + 1)
4. Determine the different configurations that influence the synthesis of the considered variable
   - by default : the number of subsets of predecessors
   - if information about cooperation is available : multiplexes...
5. for each *interesting parameterization*
   - construct the dynamics
   - retain it only if there is no contradiction
6. Results : $\{\text{model } M | dyn(M) \text{ does not present a contradiction}\}$

Application to FFL (Interaction graph & levels)

1. Graph of schematic interactions, number of levels of each variable



Application to FFL (number of models)

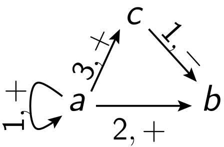
2. How many way to give values to parameters ?

$$c \in [0,1] \Rightarrow \begin{cases} K_{c,\emptyset} & \in [0,1] \\ K_{c,\{a\}} & \in [0,1] \end{cases} \qquad \text{if } a \in [0,1] \Rightarrow \begin{cases} K_{a,\emptyset} & \in [0,1] \\ K_{a,\{a\}} & \in [0,1] \end{cases}$$

$$b \in [0,1] \Rightarrow \begin{cases} K_{b,\emptyset} & \in [0,1] \\ K_{b,\{a\}} & \in [0,1] \\ K_{b,\{c\}} & \in [0,1] \\ K_{b,\{a,c\}} & \in [0,1] \end{cases} \qquad \begin{matrix} \text{if } a \in [0,2] \Rightarrow \begin{cases} K_{a,\emptyset} & \in [0,2] \\ K_{a,\{a\}} & \in [0,2] \end{cases} \\ \text{if } a \in [0,3] \Rightarrow \begin{cases} K_{a,\emptyset} & \in [0,3] \\ K_{a,\{a\}} & \in [0,3] \end{cases} \end{matrix}$$

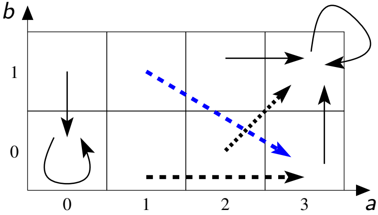
$$|\text{models}| = (2^2) \times (2^4) \times (2^2 + 3^2 + 4^2) = \mathbf{1856}$$

Application to FFL (State graph in the plane ($c = 0$))

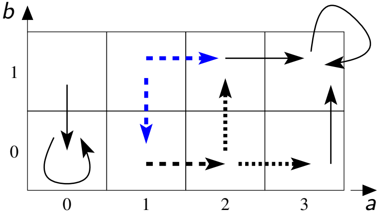


$$
\begin{aligned}
K_{a,\emptyset} &= 0\\
K_{a,\{a\}} &= 3\\
K_{b,\emptyset} &= 0\\
K_{b,\{a\}} &= 0\\
K_{b,\{c\}} &= 0\\
K_{b,\{a,c\}} &= 1\\
K_{c,\emptyset} &= 0\\
K_{c,\{a\}} &= 1
\end{aligned}
$$

a	b	c	$\omega(a)$	$\omega(b)$	$k_{a,\omega(a)}$	$k_{b,\omega(b)}$
0	0	0	$\{\}$	$\{c\}$	0	0
1	0	0	$\{a\}$	$\{c\}$	3	0
2	0	0	$\{a\}$	$\{a,c\}$	3	1
3	0	0	$\{a\}$	$\{a,c\}$	3	1
0	1	0	$\{\}$	$\{c\}$	0	0
1	1	0	$\{a\}$	$\{c\}$	3	0
2	1	0	$\{a\}$	$\{a,c\}$	3	1
3	1	0	$\{a\}$	$\{a,c\}$	3	1



synchronous state graph



asynchronous state graph

Relationship between Thomas' & differential Frameworks

- $K_{x,\omega}$: position (discrete space) of the focal point of the ODE :

$$\frac{dx}{dt} = (k_x + \sum_{y \in \omega} k_{x,y}) - \gamma_x x \qquad \Rightarrow \qquad x_{eq} = \frac{(k_x + \sum_{y \in \omega} k_{x,y})}{\gamma_x}$$

- x_{eq} has to be inside the corresponding interval
- Snoussi's constraints (monotonicity) :

$$\omega_1 \subseteq \omega_2 \qquad \Rightarrow \qquad K_{x,\omega_1} \leq K_{x,\omega_2} \qquad (k_{x,y} : \text{positive number})$$

Parameter Identification Problem (discrete modelling)

- For current example : **256** + **576** + **1024** = **1856**
- Number of parameterizations : $\Pi_{v \in V}(|G^+(v)| + 1)^{2^{|G^-(v)|}}$
- If considering only monotonous parameterizations :
  - for c : 3 different parameterizations
  - for b : 4 different parameterizations
  - for a :

$$\begin{cases} \text{if } a \in [0,1] & 3 & \text{parameterizations} \\ \text{if } a \in [0,2] & 6 & \text{parameterizations} \\ \text{if } a \in [0,3] & 10 & \text{parameterizations} \end{cases}$$

$\Rightarrow$ **36** + **72** + **120** = **228**

- But this constraint can be relaxed...
  two activators when both present can have no action because of possible complexation...
- which dynamics have to be considered ?

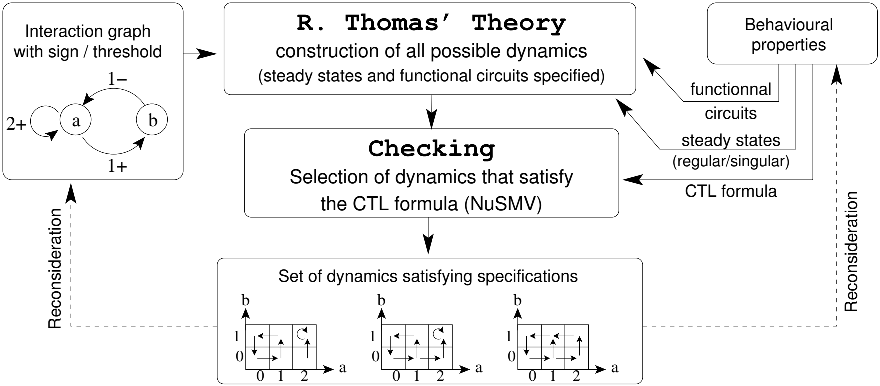
Taking into account information about the behavior

Which class of models is interesting ?

1. models which are coherent with continuous framework
2. models which present homeostasis or multi-stationarities (functionality of circuits, characteristic states)
3. models whose dynamics is coherent with known properties
   - (non-) accessibility
   - periodicity
   - efficiency of a regulation / pathway
   - temporal logic formulae (CTL for example)

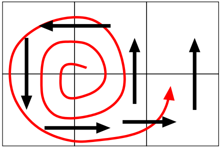
   $\Rightarrow$ automation

Automation

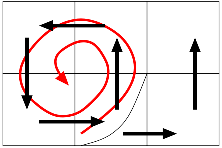

Interaction graph
with sign / threshold
1−
2+
a
b
1+
R. Thomas' Theory
construction of all possible dynamics
(steady states and functional circuits specified)
Behavioural
properties
functionnal
circuits
steady states
(regular/singular)
CTL formula
Checking
Selection of dynamics that satisfy
the CTL formula (NuSMV)
Reconsideration
Reconsideration
Set of dynamics satisfying specifications
b
1
0
0 1 2
a

Limitations

- Computational wasteful $\Longrightarrow$ Constraint based methods
- Discrete models are not able to handle « measured time »
  - Time necessary for the system to go from one state to another one is often experimentally available.
  - Time used by the system to cover a whole turn of a periodic trajectory (e.g. circadian cycle) is often available.
- Non-determinism is sometimes excessive



Outgoing Spiral

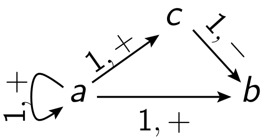


Incoming spiral

Hybrid Modeling

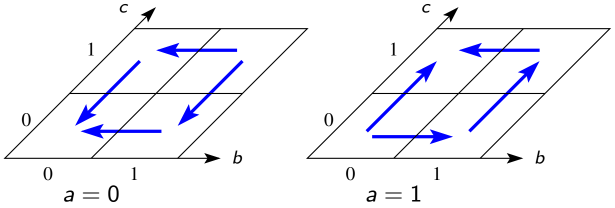
- Notion of delays of activation/inhibition
  1. when an order of activation/inhibition arrives, the biological machinery starts to increase/decrease the corresponding protein concentration,
  2. but this action takes time. $\Longrightarrow$ Clocks
  3. $d_v^+(x)$: delay to pass from level x to $x+1$
     $d_v^-(x)$: delay to pass from level x to $x-1$
- linear hybrid automata are well suited to allow such refinement.
  1. discrete states, transitions
  2. additional variables : linear evolution inside each discrete state.
  3. Idea : thresholds are no longer instantaneously triggered.
     $\Rightarrow$ New parameters : delays mandatory to cross the threshold

Example : FFL controled by a positive auto-regulation



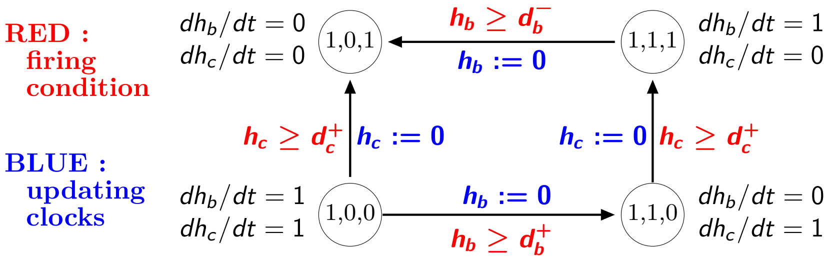
functionality of $a \longrightarrow a$:	$\begin{cases} K_a = 0 \\ K_{a,\{a\}} = 1 \end{cases}$
functionality of $a \longrightarrow c$:	$\begin{cases} K_c = 0 \\ K_{c,\{a\}} = 1 \end{cases}$
c inhibits b even if $a = 1$:	$\begin{cases} K_b = 0 \\ K_{b,\{a\}} = 0 \end{cases}$
functionality of $a \longrightarrow b$:	$\{ K_{b,\{a,c\}} = 1$
if $a = 0$, b is inhibited :	$\{ K_{b,\{c\}} = 0$

Dynamics :



First Modeling (due to R. Thomas)

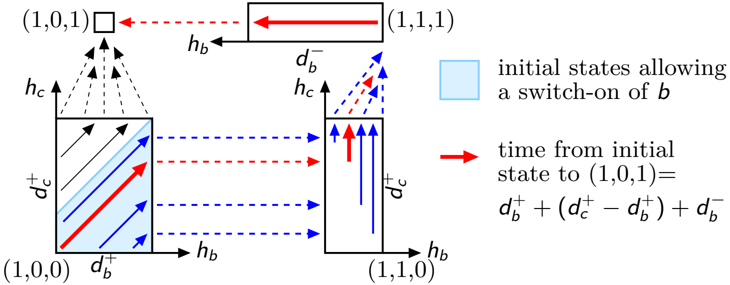
- hybrid automaton – in the plane $(a = 1)$:



- initial state : $(1, 0, 0)$ with $h_b = h_c = 0$
- if $d_c < d_b$, b will never be switched on
- if $d_c > d_b$, c gives b the time to be switched on

First Modeling (due to R. Thomas)

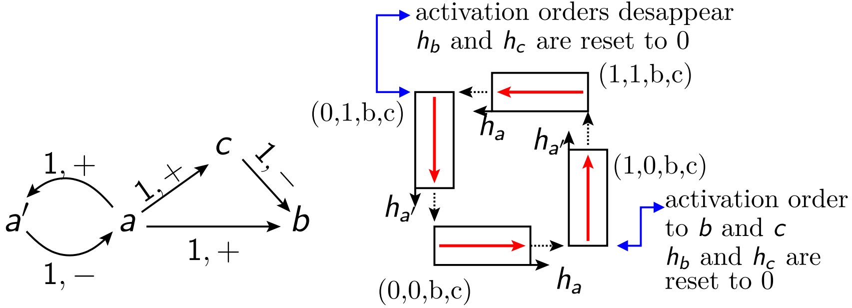
- influence of the initial state :



hypothesis	**constraints**
starting from $(1,0,0)$ with $h_b = h_c = 0$, switch-on of b is observed	$d_c^+ > d_b^+$
trajectory $(1,0,0) \rightarrow (1,1,0) \rightarrow (1,1,1) \rightarrow (1,0,1)$ takes n seconds	$d_c^+ + d_b^- = n$

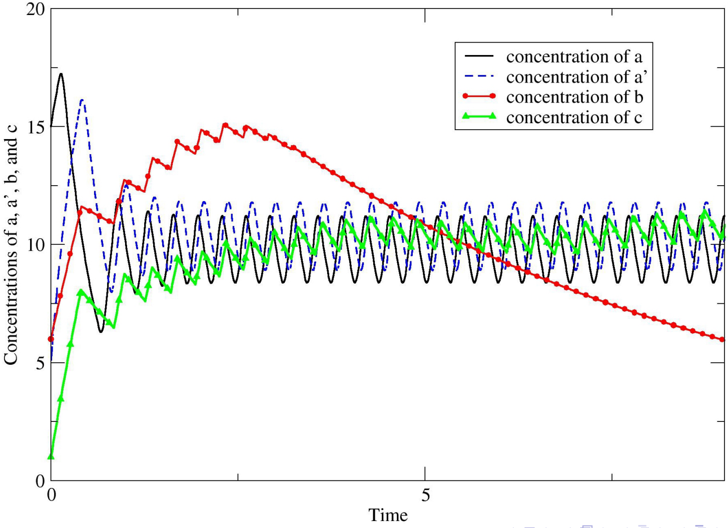
Drawback of this modeling framework

- FFL controled by a (functional) negative circuit



- accumulation is not possible

Accumulation is possible in the differential framework

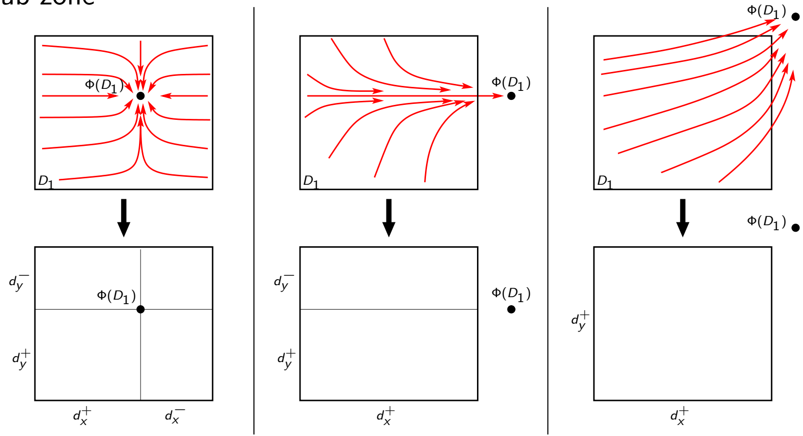


Hybrid models inspired by Differential models

- Main idea : *express a relationship between delays of the hybrid model and the differential model*
  - $d_v^+(\mu)$ is an approximation of the time necessary to variable v to cross the domain from left to right.
  - $d_v^-(\mu)$ is an approximation of the time necessary to variable v to cross the domain from right to left.
- From differential models to hybrid models :
  - thresholds are given by the differntial equations
  - parameters $K_{\ldots}$ are given by the discretization of focal points
  - delays are deducible :
    - in each domain, the differential model has an analytic solution
    - the time necessary to cross a domain is computable.

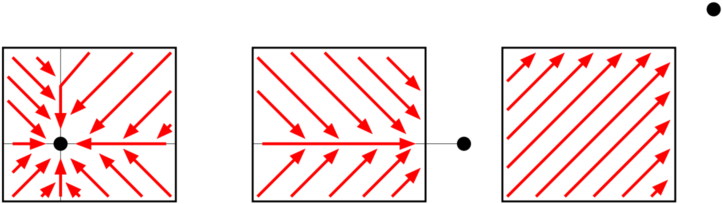
Hybrid models inspired by Diff. models : sketch (1)

- with each domain is associated a *temporal zone* which is divided into sub-zone



Hybrid models inspired by Diff. models : sketch (2)

- inside a sub-domain : **continuous linear evolution**

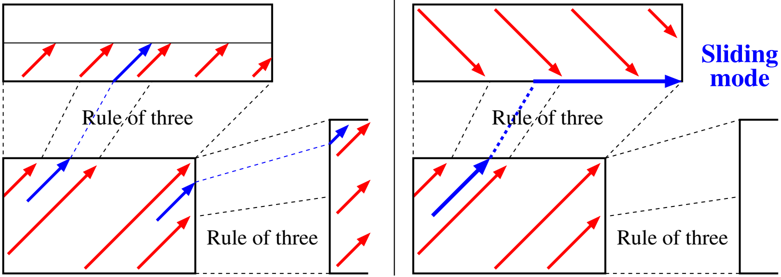


- $\Longrightarrow$ trajectories are approximated by polylines

Hybrid models inspired by Diff. models : sketch (3)

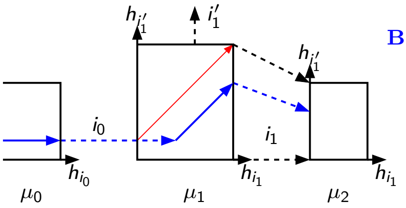
- transitions between domains :

if target temporal zone is compatible	if target temporal zone does not accept entering trajectories



Building constraints on delays from known trajectories

- Is it possible to build constraints on delays in order to make possible a trajectory passing through a given sequence of domains ?
- Principle : enumeration of constraints due to paths of length 2
- 12 situations
- example $\mu_0 \xrightarrow{i_0} \mu_1 \xrightarrow{i_1} \mu_2$:



Blue trajectory is possible :

$$(d^+_{i_1}(\mu_1) - clock_{i_1}) < (d^+_{i'_1}(\mu_1) - clock_{i'_1})$$

Constraints on the FFL with positive auto-regulation

- *b* is switched-on before *c* :
  $(1,0,0) \rightarrow (2,0,0) \rightarrow (3,0,0) \rightarrow (3,1,0) \rightarrow (3,1,1) \rightarrow (3,0,1)$

1. From $(2,0,0)$, 2 possible successors : $(3,0,0)$ and $(2,0,1)$. Considering that clocks are reset to 0 when entering into $(2,0,0)$:

$$\boldsymbol{d_a^+((2,0,0)) < d_c^+((2,0,0))}$$

2. From $\mathbf{(3,0,0)}$, 2 possible successors : $\mathbf{(3,1,0)}$ and $\mathbf{(3,0,1)}$.

$$\boldsymbol{d_b^+((3,0,0)) < d_c^+((3,0,0)) - d_a^+((2,0,0))}$$

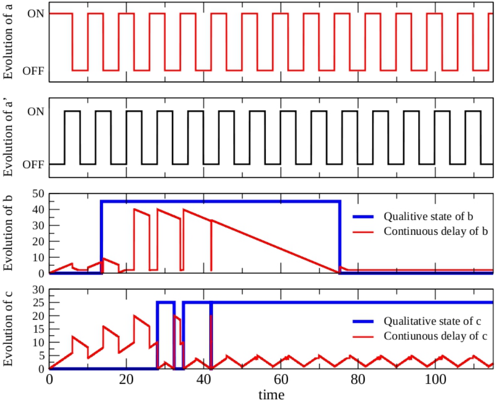
($\boldsymbol{c}$ has begun to increase)

3. From $\mathbf{(3,1,0)}$, there exists a unique successor $\Longrightarrow$ **No constraint.**
4. From $\mathbf{(3,1,1)}$, there exists a unique successor $\Longrightarrow$ **No constraint.**

Is accumulation possible in such models ? (FFL controled by a negative circuit)

Simulation of a hybrid model for the FFL controled by a negative circuit.
Initial state : $(1,0,0,0)$, initial clocks : $(2.,0.,0.,0.)$.

$d^+_{a,0,\{a'\}}$	$= 4$	$d^+_{a',0,\{a\}}$	$= 4$
$d^+_{a,0,\emptyset}$	$= 1$	$d^+_{a',0,\emptyset}$	$= 1$
$d^-_{a,0,\emptyset}$	$= 1$	$d^-_{a',0,\emptyset}$	$= 1$
$d^+_{a,1,\{a'\}}$	$= 1$	$d^+_{a',1,\{a\}}$	$= 1$
$d^-_{a,1,\{a'\}}$	$= 1$	$d^-_{a',1,\{a\}}$	$= 1$
$d^-_{a,1,\emptyset}$	$= 4$	$d^-_{a',1,\emptyset}$	$= 4$
$d^+_{b,0,\{a,c\}}$	$= 7$	$d^+_{c,0,\{a\}}$	$= 10$
$d^+_{b,0,\emptyset}$	$= 2$	$d^+_{c,0,\emptyset}$	$= 4$
$d^-_{b,0,\emptyset}$	$= 2$	$d^-_{c,0,\emptyset}$	$= 16$
$d^+_{b,1,\{a,c\}}$	$= 2$	$d^+_{c,1,\{a\}}$	$= 4$
$d^-_{b,1,\{a,c\}}$	$= 2$	$d^-_{c,1,\{a\}}$	$= 4$
$d^-_{b,1,\emptyset}$	$= 80$	$d^-_{c,1,\emptyset}$	$= 10$



Conclusion

- Parameter identification problem
- This step depends on the information the modeler put into the model
  - possible automation for discrete models
  - no automation for differential models
- Properties with elapsed time are available :
  - Discrete models do not take into account elapsed time
  - Differential models do, but difficulty for model-checking
- Hybrid models can fill up the gap between discrete models and differential ones.

Open questions

1. automation of the transcription of dynamical properties
2. enumeration of the parameter valuations compatible with reachability properties (hybrid constraints programming)
3. model checking between a real time property and a hybrid model (combinatorial explosure / symbolic model-checking).
4. hybrid formal logic for constraining parameters (Berlin)

$\Rightarrow$ Aim : predictions $\Longrightarrow$ (discriminating) biological experiments

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– Thanks for your attention –